

# PATENT SPECIFICATION

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## Separation of Sterols.

### COMPLETE SPECIFICATION

- We, EASTMAN KODAK COMPANY, a Company organized under the Laws of the State of New Jersey, United States of America, of 343, State Street, Rochester 4, New York, United States of America (Assignees of WINTON BROWN and HERBERT WILLIAM RAWLINGS) do hereby declare the invention for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:
- This invention concerns a process for separating sterols, and more particularly, to a process for separating sterols from deodorizer sludges.
- Sterols are desired materials of commerce since they are used in the preparation of such medicinals as cortisone and sex hormones, as well as for starting materials in the syntheses of other products. Deodorizer sludges and other concentrates of unsaponifiable materials resulting as by-products in the processing of fats and oils are the common source of sterol materials. The usual method for recovering sterol materials from such fatty materials containing substantial amounts of sterol materials is to dissolve the fatty material in a solvent such as acetone or methyl ethyl ketone and to cool the resulting mixture to about  $-20^{\circ}\text{C}$ . to winter out the sterol materials. However, the sterol products resulting from such wintering processes are of low purity, as substantial amounts of such oleaginous material as glycerides, fatty acids and tocopherols are also precipitated therewith. Further, such wintered products are mixtures of sterols and sterol esters. Hence, such wintered products must be subjected to further processing and crystallization or purification to prepare a desirable sterol product.
- Several methods for separating sterols from such materials as deodorizer sludges have been proposed. However, such methods commonly are comprised of numerous and frequently costly or time-consuming processing steps, they produce mixtures of sterols and sterol esters and are low-yielding or produce low purity sterols.
- It would thus be desirable to use an improved process for separating sterols from concentrates of sterol materials in high purity and in high yields with a minimum of processing steps.
- According to the present invention, there is provided a process for the separation of sterols from fatty materials containing a substantial amount of sterol material which comprises saponifying the fatty material in a methanolic alkaline medium, acidulating the resulting saponified mixture to convert the resulting soaps to free fatty acids, whereby a glycerol-containing aqueous phase and a sterol-containing phase are formed, separating the two phases, dissolving the sterol-containing phase at a raised temperature in a methanolic solvent medium, cooling the resulting solution to a temperature from  $0^{\circ}$  to  $20^{\circ}\text{C}$  to selectively crystallise sterols therefrom, and finally separating the crystallised sterols from the said solution.
- It is found that the present invention enables sterols to be prepared from sterol-containing fatty materials in high purity and in high yields with a reduced number of processing steps. The invention is particularly suitable for separating sterols from deodorizer sludges and frequently gives sterols in high yields in a purity of over 90%. Further, the amount of cooling required is less than is employed in previously proposed processes for the separation of sterols.
- Any fatty material containing a substantial amount of sterol material may be employed in the present process. Animal and vegetable oils contain relatively low concentrations of sterols and are thus not usually employed as sources of sterols as

such. However, concentrates of sterols commonly result from the processing of animal and vegetable oils which concentrates are desirable starting materials in the present process, both from the standpoint of sterol concentration and cost. Typical of such materials are deodorizer sludges, fatty acid distillation residues, still bottoms such as tall oil still bottoms and soap stocks. The preferred starting materials in the subject process are deodorizer sludges.

Deodorizer sludges are also called "hot-well scum", "lighter than water scum", "clabber stock", "condenser oil" and "catch basin scum", all such materials being usable in this invention and included by the generic term "deodorizer sludge". Deodorizer sludges are by-products resulting from the deodorization of fatty oils with steam. The by-product sludge is usually separated from the steam in traps, condensers or similar means of separation. Deodorizer sludges derived from such vegetable oils as soybean oil, cottonseed oil, corn oil, safflower oil, and similar fatty oils can be suitably employed in the present process. Deodorizer sludges are mixtures of several oleaginous materials including sterols, glycerides, free fatty acids and tocopherols, as well as small amounts of miscellaneous organic and inorganic materials including soaps, polymeric fatty acids and polymeric tocopherols. The sterol component in deodorizer sludges is a mixture of unesterified or free sterols and sterol esters. Deodorizer sludges usually contain from about 5-10% by weight of sterols, although some deodorizer sludges contain as much as 20% or even 25% by weight of sterols.

In carrying out the present process, a fatty material containing a substantial amount of sterol material, such as a deodorizer sludge, is initially subjected to saponification in a methanolic alkaline medium to split the fatty acid esters. The saponification may be effected with any of the commonly used alkaline saponifying agents such as an alkali metal hydroxide, with sodium and potassium hydroxide being preferred. The saponification is typically effected by mixing the alkaline saponifying agent, deodorizer sludge and methanol, and thereafter heating the resulting mixture at an elevated temperature, more usually at the reflux temperature of the methanol, to substantially completely saponify the esters.

The resulting saponified mixture is thereafter acidulated to convert the resulting soaps to free fatty acids. Substantial excesses of acid over that required to decompose the soaps are to be avoided. Thus, sufficient sulphuric acid may be added to convert the soaps to free fatty acids but in amounts less than is required to convert the alkali to a bisulphate.

The acidulation of the saponified mixture results in a composition composed primarily of free fatty acids, tocopherols, unesterified or free sterols, glycerol, water, methanol, and alkaline metal salts of the neutralizing acid. This mixture forms two phases, namely, an oleaginous or organic phase containing the unesterified sterols, free fatty acids, part of the methanol and tocopherols, and an aqueous phase containing glycerol, water, part of the methanol, neutralizing acid and salts. The removal of the aqueous or glycerol-containing phase from the organic phase can be easily effected as the aqueous phase settles to the bottom of the reaction mixture and can be drawn off.

The methanol remaining dissolved in the organic phase serves to repress back-esterification of the sterols with the free fatty acids in this phase. The separation of the glycerol-containing phase from the organic phase is desirably effected at elevated temperatures between about 30°C. and the reflux temperature of the methanol so that sterols do not crystallize out during this step. In practice it is convenient to effect the methanolic saponification at reflux temperatures and to successively effect the acidulation and phase separation steps directly on the heated saponification reaction mixture.

The separated sterol-containing fatty acid composition is preferably dissolved in a methanol-containing solvent medium composed of 9-100% by volume of methanol, 0-90% by volume of acetone or methyl ethyl ketone, and 0-10% by volume of water. The solvent medium is thereafter cooled, and the free sterols selectively crystallized and separated therefrom as described hereinafter. A particularly effective methanol-containing solvent medium is composed of: (1) 9-40%, and preferably 14-25%, by volume of methanol; (2) 59-90%, and preferably 70-85%, by volume of acetone or methyl ethyl ketone; and (3) 1-10% by volume of water. Another effective solvent medium is one containing 90-100% by volume of methanol and 0-10% by volume of water.

The separated sterol-containing fatty acid composition is dissolved in the methanol-containing solvent medium at an elevated temperature up to the reflux temperature of the solvent medium. Concentrations of 1 gram of the separated sterol-containing fatty acid composition for each 1-5 ccs. of methanolic solvent are usually preferable, although concentrations of 1 gram of sterol-containing fatty acid composition for each 1-10 ccs. of solvent can be employed if desired. Thereafter, the resulting solution is cooled to selectively crystallize out the sterols, particularly high yields and high purities of crystallized sterol being obtained at crystallization temperatures of 0°-20°C. Filtration is usually employed to separate

the fractionally crystallized sterols from the mother liquor, although any of the other well-known methods of separating solids from liquids such as decanting, and centrifuging can also be utilized.

The presence of methanol in the reaction mixture during the successive processing steps of the present invention is particularly important as the methanol serves to repress a back-esterification of the sterols. In the absence of methanol, a mixture of sterols and free fatty acids tends to esterify in part to produce a mixture of sterols and sterol esters. Such mixtures of partially esterified sterols are difficult to selectively crystallize in high purity and in high yields from other oleaginous materials such as free fatty acids and tocopherols usually in admixture therewith. However, in the present process where methanol is present during each successive step, there is substantially no back-esterification of sterols with the free fatty acids, and the sterols can be readily selectively separated from the reaction mixture in high purity and in high yields.

The invention is illustrated by the following examples.

#### EXAMPLE 1.

Sterols were separated by the present process from a deodorizer sludge prepared by the steam deodorization of soybean oil and which contained about 35% by weight of glycerides, about 25% by weight of free fatty acids, 10% by weight of tocopherols, about 19.2% by weight of sterols based on an infra-red assay of a saponified sample, and about 9.8% of miscellaneous organic and inorganic materials. A 2.5 kilogram sample of the deodorizer sludge was refluxed for one hour with 11.30 ccs. of methanol, 53 ccs. of water and 456 grams of 50% aqueous sodium hydroxide. Thereafter the resulting soaps were split by adding 1320 grams of 25% sulphuric acid to the reaction

mixture and by heating at a temperature just below the reflux temperature of the mixture until an oil layer containing the sterols, fatty acids and tocopherols formed on top of an aqueous layer in the reaction mixture. Approximately 3 gallons of hot water (about 70°C.) were then added and the aqueous or glycerol-containing layer was drawn off and discarded. The remaining oil layer, or sterol-containing fatty acid composition, was washed once again with 2 gallons of hot water and phase separated as before. The fatty acid composition was then dissolved by refluxing in a solvent medium composed of 5800 ccs. of acetone, 1140 ccs. of methyl alcohol and 150 ccs. of water. The resulting solution was cooled to 4°C. and left at that temperature for 16 hours and thereafter filtered through a Buchner funnel. The filter cake was then washed with 500 ccs. of the crystallizing solvent medium at 4°C. The washed filter cake was then vacuum dried at about 80°C. to yield 495 grams of material assaying 92.5% by weight of sterols by infra-red assay for a yield of 95.3%.

#### EXAMPLE 2.

A. In accordance with the method described in Example 1, a sterol-containing fatty acid composition was prepared by saponifying and acidulating a deodorizer sludge of the type described in Example 1. Several samples of the resulting sterol-containing fatty acid composition were thereafter dissolved in various solvent media of the present invention at concentrations of 3 ccs. of solvent per gram of sterol-containing fatty acid composition, crystallized and separated from the solvent by the methods described in Example 1. Table 1 below summarizes the results of these crystallizations. The proportions of solvents in Table 1 are expressed in terms of percent by volume.

TABLE 1

	Solvent Medium	Crystallization Temperature	Purity By Infra-red	Yield By Infra-red
90	a) methanol	4°C.	91.5%	92.7%
	b) 95% methanol + 5% water	4°C.	91.5%	89.5%
95	c) 77% acetone + 19% methanol + 4% water	4°C.	96.9%	87.8%
	d) 77% methyl ethyl ketone + 19% methanol + 4% water	4°C.	97.5%	84.7%
	e) methanol	20°C.	89.5%	84.0%
100	f) 95% methanol + 5% water	20°C.	94.5%	75.5%
	g) 77% acetone + 19% methanol + 4% water	20°C.	93.0%	74.5%
	h) 77% methyl ethyl ketone + 19% methanol + 4% water	20°C.	99.0%	66.0%
105	i) 74% acetone + 18.5% methanol + 7.5% water	20°C.	98.0%	77.2%

B. For comparison purposes, several samples of sterol-containing fatty acid composition prepared by saponifying and acidulating a deodorizer sludge of the type described in Example 1 were crystallized from several solvents not of the type employed in the present invention. The samples were dissolved in the various solvent media at concentrations of 3 ccs. of solvent per gram of sample, crystallized and separated from the solvents by the methods described in Example 1. Table 2 below summarizes the results of these crystallizations. The proportions of solvents in Table 2 are expressed in terms of percent by volume.

TABLE 2

Solvent Medium	Crystallization Temperature	Purity By Infra-red	Yield By Infra-red
a) acetone	-20°C.	47%	59.3%
b) methyl ethyl ketone	-20°C.	60%	86.0%
c) acetone	4°C.	60%	13.7%
d) methyl ethyl ketone	4°C.	70%	2.5%
e) acetone	20°C.	(no crystallization)	
f) methyl ethyl ketone	20°C.	(no crystallization)	

The above examples illustrate that the present process can be employed to prepare sterols in high purity and in high yields with a minimum of process steps. The above examples also show the superiority of the present methanol-containing solvents in the present process as distinguished from conventional solvents. A practical operational advantage of the present process is also illustrated by the examples in that the sub-zero wintering temperatures usually used for separating sterol materials from other oleaginous materials are not needed as sterols can be efficiently crystallized and separated at room temperatures, such as at about 20°C. Accordingly, refrigeration costs are substantially lower in the present process. Thus, the present process provides a particularly efficient method for separating sterols from fatty materials containing substantial amounts of sterols such as deodorizer sludges.

## WHAT WE CLAIM IS:

1. A process for the separation of sterols from fatty materials containing a substantial amount of sterol material which comprises saponifying the fatty material in a methanolic alkaline medium, acidulating the resulting saponified mixture to convert the resulting soaps to free fatty acids, whereby a glycerol-containing aqueous phase and a sterol-containing phase are formed, separating the two phases, dissolving the sterol-containing phase at a raised temperature in a methanolic solvent medium, cooling the resulting solution to a temperature from 0° to 20°C to selectively crystallize sterols therefrom, and finally separating the crystallized sterols from the said solution.

2. A process as claimed in Claim 1, in which the methanolic solvent medium consists of 9-100% by volume of methanol, 0-90% by volume of acetone or methyl ethyl ketone, and 0-10% by volume of water.

3. A process as claimed in Claim 2, in which the methanolic solvent medium consists of 90-100% by volume of methanol and 0-10% by volume of water.

4. A process as claimed in Claim 2, in which the methanolic solvent medium consists of 59-90% by volume of acetone or methyl ethyl ketone, 9-40% by volume of methanol and 1-10% by volume of water.

5. A process as claimed in Claim 4, in which the methanolic solvent medium consists of 70-85% by volume of acetone or methyl ethyl ketone, 14-25% by volume of methanol, and 1-10% by volume of water.

6. A process as claimed in any preceding claim, in which 1 gram of sterol containing phase is dissolved in from 1 to 5 ccs. of methanolic solvent medium.

7. A process as claimed in any preceding claim, in which the fatty material employed in the process is deodorizer sludge.

8. A process as claimed in any preceding claim, in which the acidulation is effected by the addition of sulphuric acid.

9. A process for the separation of sterols according to Claim 1 substantially as herein described.

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